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# Sphingolipids of the Stratum Corneum and Lamellar Granules of Fetal Rat Epidemis

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**Sphingolipid profiles have been determined for whole epidermis, a subcellular fraction enriched in lamellar granules, and a fraction enriched with stratum corneum derived from fetal rat skin. In each case, 4 groups of glucosylceramides and 6 groups of ceramides have been identified by thin-layer chromatographic comparison with structurally defined sphingolipids from pig epidermis. The relative amounts of the sphingolipids in each preparation have been quantified by photodensitometry of the charred chromatograms. Lamellar granule sphingolipids had elevated proportions, relative to whole epidermis, of acylceramides, acylglucosylceramides, and a glucosylceramide fraction which may be produced by *O*-deacylation of the acylglucosylceramides. The fetal stratum corneum-enriched samples contain reduced proportions of all glucosylceramides and acylceramides as compared to lamellar granule lipids. The possible functions of these sphingolipids in the assembly and structure of lamellar granules are discussed.**

The barrier to water in stratum corneum appears to reside in the extracellular materials that fill the spaces between the cells of the stratum corneum [1–4]. This material, which forms broad membranous sheets, consists principally of lipids and is thought to arise from the contents of lamellar granules extruded from epidermal cells [4,5]. Although the ultrastructure and histochemistry of both the stratum corneum and the lamellar granules have now been extensively studied, only stratum corneum has been previously subjected to detailed lipid and protein analysis. Lamellar granules have only recently been isolated in partially purified form [6,7] and studies of their lipid content are still preliminary [7,8].

Stratum corneum lipids consist principally of cholesterol, free fatty acids, and ceramides, with only small amounts of glycolipid and little if any phospholipid. The ceramides have been of considerable interest because this structurally heterogeneous group represents the major polar lipids from which the extracellular membranous structures of the stratum corneum are constructed. Although the structural heterogeneity among the ceramides was indicated by the earlier work of Gray and White [9], it was only recently that the individual structures of the epidermal ceramides were elucidated [10]. The most unusual of these ceramides is an acylceramide which contains a high proportion of esterified linoleic acid and an amide-linked hydroxyacid. The hydroxyacid moiety is of sufficient length to completely span a typical unit membrane, allowing the linoleate to extend into an adjacent bilayer.

In essential fatty acid deficiency, abnormalities of structure and function in the epidermis include a defective water barrier. This latter defect appears to be due to a direct requirement for linoleic acid independent of its role as a precursor for prostanoid synthesis [11–13]. In essential fatty acid-deficient epidermis, the lamellar granules lack internal structure and the extracellular sheets of the stratum corneum appear to be sparse and fragmentary [14]. The acylceramide and the structurally related acylglucosylceramide [15,16], which are carriers of linoleate, have been postulated to carry out membrane-organizing and stacking functions [10,17].

In the present investigation, the ceramides and glucosylceramides from fetal rat epidermis have been examined by quantitative thin-layer chromatography. Lipid profiles have been determined for whole epidermis, a fraction enriched in stratum corneum, and a fraction of partially purified lamellar granules in order to examine the relative content of these lipids and their relationship to function of the skin.

## MATERIALS AND METHODS

### *Fetal Rat Skin Fractions*

Pregnant Sprague-Dawley (Holtzman) rats were sacrificed at 20 days of gestation. Whole fetal epidermis and a lamellar granule-enriched fraction were prepared as previously described [6,18]. Briefly, epidermis was separated from whole skins after incubation with 0.1 M dithiothreitol, and lamellar granules were purified on the basis of buoyant density by metrizamide-gradient centrifugation. A stratum corneum-enriched preparation was obtained from full-thickness fetal epidermis by trypsinization as previously described [19]. Samples homogenized in 0.25 M sucrose were kept frozen on dry ice prior to further processing and lipid extraction as described below.

### *Lipid Extraction*

Samples representing approximately 4 g of whole fetal epidermis were quickly thawed and transferred to 25-ml Ehrlenmeyer flasks. These samples were refrozen and taken to dryness by lyophilization. The dried material was extracted successively with 10-ml portions of chloroform:methanol, 2:1, 1:1, and 1:2. Each extraction was conducted at room temperature for a period of 2 h. Combined extracts were filtered through a cotton plug, taken to dryness under a stream of nitrogen, and redissolved in 5 ml of chloroform:methanol, 2:1. To this solution, 1 ml of water was added [20]. The resulting lower phase was transferred to a second tube and washed with an additional 3 ml of theoretical upper phase [20]. The combined upper phases and the lower chloroform phase were taken to dryness under nitrogen. Final drying was achieved in vacuo. The upper phase was found to contain only sucrose and metrizamide. The lower phase, which contained the lipids, was redissolved at a concentration of 10 mg/ml of chloroform:methanol, 1:1, and analyzed by thin-layer chromatography as described in the following section.

### *Thin-Layer Chromatography*

All thin-layer chromatography employed 20 × 20 cm glass plates coated with a 0.5 mm-thick layer of silica gel 60H (E. M. Reagents, Darmstadt, West Germany). Plates were activated at 110°C, washed with chloroform:methanol, 2:1, and scored into 6 mm-wide lanes prior to use. One sample or standard was applied to each lane. Development with chloroform:methanol:water, 40:10:1, resolved 4 groups of glucosylceramides from the combined ceramides plus nonpolar lipids. The individual ceramide fractions were resolved and separated from the nonpolar materials by development of a second plate with chloro-

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form:methanol:acetic acid, 190:9:1. After solvent development, the chromatograms were air dried, sprayed with 50% sulfuric acid, and charred on a 220°C hotplate. The charred lipids were quantitated by photodensitometry as previously described [21,22].

*Identification of Sphingolipids*

Four glucosylceramides and 6 ceramide fractions were recently isolated from pig epidermis and are now structurally defined [10,16,23]. These materials served as thin-layer chromatographic standards for the identification of the fetal rat sphingolipids. Also, glucosylceramides were noted to produce a characteristic red-violet color prior to charring when heated in the presence of sulfuric acid. Identities of the *O*-acylceramides and *O*-acylglucosylceramides were supported by chromatography before and after a mild saponification. Samples were saponified by treatment with 1 M methanolic potassium hydroxide for 1 h at 60°C. After neutralization with HCl, products were extracted into chloroform, concentrated and compared with the original lipid mixture on thin-layer chromatography. Saponification converts acylglucosylceramide to a fraction C glucosylceramide [23], while acylceramide (ceramide fraction 1) reacts to produce a fraction 3 ceramide [10].

## RESULTS

Sphingolipid compositions found for whole fetal rat epidermis, a lamellar granule-enriched preparation, and a fetal stratum corneum-enriched fraction are summarized in Table I. Four series of glucosylceramides and 6 series of ceramides have been identified by thin-layer chromatographic comparison with previously characterized pig epidermal sphingolipids [10,16, 23]. Acylglucosylceramide (glucosylceramide A) and acylceramide (ceramide 1) identities were supported by the fact that these materials react during a mild saponification, whereas the other sphingolipids are unreactive.

Comparing the lamellar granule sphingolipid profile with that of whole epidermis, it is apparent that lamellar granule lipids contain higher proportions of glucosylceramides A and C and ceramide 1. In accord with earlier reports on lipid composition of different epidermal strata [19,24], the stratum corneum-enriched preparation contains only small amounts of glucosylceramides relative to ceramides. These glycolipids may be contributed by the incompletely cornified outer layers of the fetal stratum corneum [25]. Also, the stratum corneum contains a lower proportion of ceramide 1 and a higher proportion of ceramide 3 as compared to lamellar granule lipid.

In Fig 1 are shown representative structures of the acylceramide and acylglucosylceramide which have previously been characterized from adult rat epidermis [26]. In the rat, a component of ceramide fraction 3 corresponds to the *O*-deacylated version of ceramide 1 [26], and glucosylceramide fraction C contains a component corresponding to an *O*-deacylated congener of the acylglucosylceramide.

TABLE I. *Sphingolipids from fetal rat epidermis*

| Sphingolipid | Lamellar granules | Whole epidermis | Stratum corneum |
|---|---|---|---|
| Glucosylceramide A (acylglucosylceramide) | 7.8[a] | 4.0 | 1.6 |
| Glucosylceramide B | 2.9 | 3.5 | 1.2 |
| Glucosylceramide C | 8.8 | 1.2 | 0.4 |
| Glucosylceramide D | tr | tr | tr |
| Ceramide 1 (acylceramide) | 32.3 | 19.3 | 15.8 |
| Ceramide 2 | 24.6 | 26.8 | 35.8 |
| Ceramide 3 | 12.9 | 24.8 | 28.6 |
| Ceramide 4 | 2.4 | 4.3 | 4.0 |
| Ceramide 5 | 6.0 | 13.6 | 11.5 |
| Ceramide 6 | 2.5 | 3.2 | 3.1 |

[a] Results are presented as weights percent. Each value represents the mean of 2 separate experiments, and average deviations from the mean were ± 20% relative. Glucosylceramides and ceramides are named in accord with previously used nomenclature [10,23].

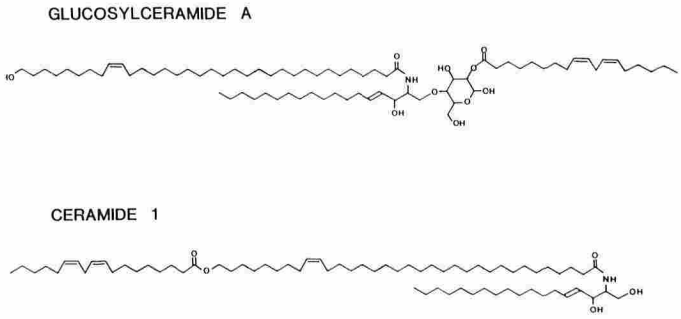


FIG 1. Structures of the acylceramide (ceramide 1) and the acylglucosylceramide (glucosylceramide A) from rat epidermis [26]. Similar molecules have been found among the epidermal sphingolipids of the horse [28] and pig [10,16]. Important features of these structures are the length of the ω-hydroxyacids and the presence of ester-linked linoleic acid.

## DISCUSSION

Indirect histochemical methods [27], as well as more direct preliminary analyses [7,8], have indicated the presence of both ceramides and glucosylceramides in epidermal lamellar bodies. However, the small amounts of materials which can be prepared by present methods and the lack of appropriate chromatographic standards have precluded more detailed analyses of these sphingolipids. In the present study, the recently characterized ceramides [10] and glucosylceramides [16,23] from pig epidermis have been used to identify similar components among the lamellar granule lipids. Thin-layer chromatographic analyses have revealed that the lamellar granules contain 4 glucosylceramide fractions and 6 ceramide fractions, each with its counterpart among the pig epidermal sphingolipids.

Of greatest interest are the acylceramides and acylglucosylceramides. The analogous materials from pig [10,16], horse [28], and adult rat epidermis [26] have now been characterized in detail. They have been shown to contain high proprotions of ester-linked linoleic acid and amide-linked long-chain ω-hydroxyacids, and it has been suggested that these structures may serve to stack up the lipid lamellae that are observed within lamellar granules [10,17]. In this regard it is of interest that the lamellar granule fraction is enriched in both of these linoleic acid-rich lipids as compared to full-thickness epidermis. The lamellar granule sphingolipids also have an elevated proportion of glucosylceramide fraction C which may arise by *O*-deacylation of the acylglucosylceramide.

It has been suggested that the lamellae of the lamellar granules may represent stacks of flattened liposomes [29]. Within this context, the possibility has been raised that the acylceramide may promote flattening of the liposomes [10] while the acylglucosylceramide may serve to hold together adjacent liposomes [17]. These suggestions were in part based upon the usual asymmetric distribution of glycolipids in membranes and upon the relative dimensions of the polar regions of these molecules. The acylglucosylceramides are capable of spanning the somewhat larger polar regions which appear to correspond to the interliposomal regions of the lamellar granules [29]. The demonstration in the present report of elevated proportions of acylceramides and acylglucosylceramides in the lamellar granule fraction supports these speculations.

It is also of interest to compare the sphingolipid profiles for lamellar granule and stratum corneum fractions. It is a striking result that the proportions of all of the glucosylceramides and ceramide 1 are reduced in going from the lamellar granule to stratum corneum, while the proportions of all of the remaining ceramides, especially ceramide fraction 3, are increased during this transition. There apparently is considerable enzymatic remodeling of the epidermal sphingolipids during or after ex-

trusion from the lamellar granule. Hydrolases specific for acylglucosylceramides have not yet been demonstrated but fractions enriched in lamellar granules do contain a variety of acid hydrolases including phospholipases [8]. Thus lipid remodeling which appears to include degradation of glycosylceramides and partial hydrolysis of ceramide 1 may occur via enzymes also packaged in lamellar granules. Nevertheless, a significant level of ceramide 1 is retained in the stratum corneum, where it may function in cementing together the extracellular lipid sheets that make up the barrier to water loss.

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